

Circadian Cycles of Lactic Dehydrogenase in Urine and Blood Plasma: Response to High Pressure

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The existence of circadian cycles of plasma lactic dehydrogenase has been confirmed. This cycle is characterized by having its lowest activity at 0800 hours and its highest activity between 1600-2000 hours. A diurnal variation in urinary excretion of lactic dehydrogenase has been described which features an inverse relationship to that of the plasma in that maximum excretion occurs during the 0800-1200 hour period while minimum excretion is present between 1600-2400 hours. High pressure stress applied to the ascending phase of the plasma cycle causes no abnormal response while the same treatment applied to the descending segment elicits a second peak of lactic dehydrogenase activity.

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THE MEASUREMENT of plasma lactic dehydrogenase activity is perhaps the most widely employed enzymatic determination used in the clinical laboratory. Elevations in plasma activity of this enzyme system are generally attributed to leakage from diseased and damaged tissue, allowing enzymes from damaged cells to be spilled into the blood stream. However, alterations in plasma lactic dehydrogenase activity do not necessarily reflect tissue dumping. Jacey and Schaefer¹ have shown that, at least in the case of acute respiratory acidosis, increased plasma lactic dehydrogenase activity is due neither to necrosis nor increased cell membrane permeability. Moreover, the existence of diurnal variations of plasma lactic dehydrogenase activity has been suggested by Starkweather and co-workers² who presented data on one subject. This cycling of lactic dehydrogenase is apparently accomplished by the influx and outflow of the M₁ form. In this report we present data which prove the presence of circadian cycles of lactic dehydrogenase in plasma.

Since lactic dehydrogenase activity has been detected in urine, although in low concentrations,⁷ a study was undertaken to determine whether urinary lactic dehydrogenase excretion was also circadian in nature.

As part of a general preparatory program for the "Sealab" series, experiments were carried out to investigate the relationship of these cycles and their response to an environmental challenge, high pressure.

METHODS

Venous blood samples from both military and civilian laboratory personnel were obtained at 4-hour intervals for 24 hours commencing at 0800 hours. The samples were collected in heparinized syringes and immediately centrifuged at 2500 g for 15 minutes at room temperature. Plasma was kept at 4°C for periods of up to 4 hours before assay. Enzyme determinations were performed utilizing the Sigma Chemical Co. ultra-violet analytical kits.

Individual urine samples were collected from the same group of subjects for one day prior to, as well as, during, the period of blood sample collection, for a total time of 48 hours. The subjects were instructed to maintain a normal diet and fluid intake and to avoid alcohol 24 hours prior to and during the experiment. Urine was voided into clean containers, the time and volume recorded and a 10 ml. aliquot frozen. An attempt was made to adhere to a 4-hour sampling schedule but this could not be rigidly maintained. Urine samples were thawed within 10 days of collection and dialyzed against cold distilled water for two hours. Urinary lactic dehydrogenase activity was measured by the same method as was plasma, except that the molarity of the buffer was increased to reflect a 1 ml. sample size.

Urine enzyme excretion results are given as percent of the 24-hour total for each four-hour sampling period. The enzyme activity (units/ml) was multiplied by the calculated excretion rate (ml/hr.) for each sample. The resulting values were plotted across bar graphs and numerically integrated over uniform intervals of 4 hours. This method of presentation was necessary because of the non-uniform voiding times of each subject.

High Pressure Protocol—Volunteer subjects who had passed pressure tests, but did not dive routinely, were divided into two groups. The first group, composed of 10 men, was exposed to pressure at 0800 hours while the second group, numbering 7, was pressurized at 2400 hours. In both cases the subjects underwent no-decompression dives to 135 feet in compressed air for 10 minutes of bottom time. Descent and ascent rates were 75 and 60 feet/minute, respectively. Blood samples were collected immediately before and after the dive, on the surface, and four and eight hours after the dive.

RESULTS

The average plasma lactic dehydrogenase level at 4-hour intervals over a 24-hour period are shown in Figure 1. It can be seen that the activity rises during the day and exhibits its highest values during the 1600-2000 hour period and declines to its lowest value

around 0800 hours. Highest activity was present at 1600 hours in 6 subjects and at 2000 hours in the other 7 subjects. All subjects displayed a 24-hour periodicity. The circadian nature of normal urinary lactic dehydrogenase

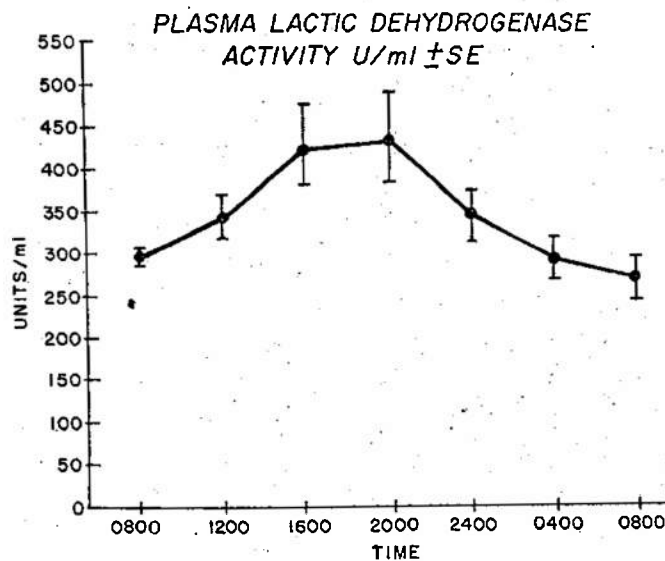


Fig. 1. Circadian cycle of plasma lactic dehydrogenase activity.

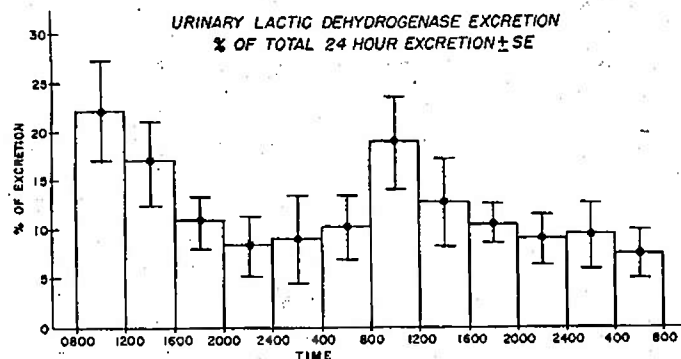


Fig. 2. Circadian cycles of urinary excretion of lactic dehydrogenase.

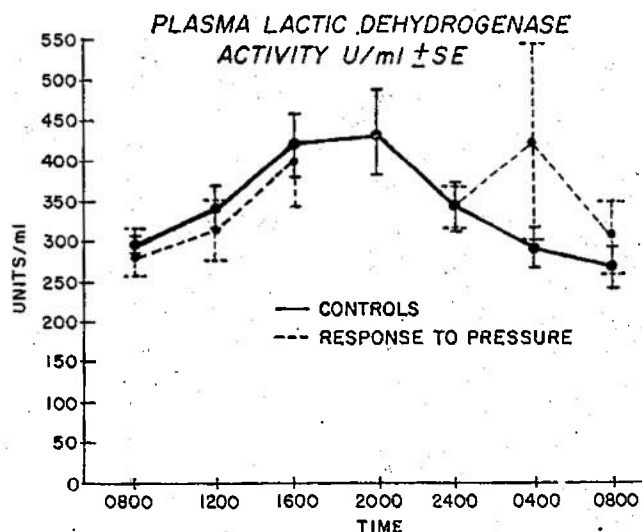


Fig. 3. Effect of high pressure at 0800 and 2400 hours upon the circadian cycle of plasma lactic dehydrogenase.

drogenase excretion is demonstrated in Figure 2. The maximal level of enzyme excretion occurs during the 0800-1200 period while minimum excretion is seen between 1600 and 2400 hours.

The response of the plasma lactic dehydrogenase system to dives, at 0800 hours and at 2400 hours, are presented in Figure 3. The results are superimposed upon the normal plasma cycle. Enzyme values immediately after the dive are not shown since they are identical to the pre-dive levels. The 0800-hour group displayed a slight increase in enzyme activity at noon and a further increase was noted at 1600. These increases correspond to those seen in the normal cycle at 1200 and 1600 hours and are assumed to reflect the ascending phase of the cycle. The men who comprised the midnight group responded in a manner directly opposite to that seen in the normal cycle. Instead of a decline in activity between 2400-0400 hours, an increase in lactic dehydrogenase activity occurred. Following this second peak at 0400 hours, a return to normal enzyme activity was seen at 0800 hours.

Peak to trough values, compared on the basis of the 24-hour average, are statistically different at the 5 percent level or better for both plasma lactic dehydrogenase activity and urinary lactic dehydrogenase excretion.

Exposure to increased pressure at 0800 hours produces no alteration in the circadian nature of plasma lactic dehydrogenase activity while the same stress applied at 2400 hours elicits an additional peak in the enzyme cycle.

DISCUSSION

Plasma lactic dehydrogenase activity exhibits diurnal variations with the lowest activity around 0800 hours and the highest activity between 1600-2000 hours. The data of Starkweather, et al.⁹ suggested a peak between 1800-2000 hours but their samples were collected every 2 hours. This circadian variation represents an increase of 50 percent in activity over the base line values during the course of a day. For this reason, elevated values for plasma lactic dehydrogenase activity obtained during the afternoon and early evening hours should be interpreted with caution when they are used as an index of pathological significance.

As the level of plasma lactic dehydrogenase starts to decline, an increase occurs in urinary excretion. The increase in plasma activity is accompanied by a decrease in urinary enzyme excretion. The mechanism of this inverse relationship has not been clarified. Crockson¹ has suggested that urinary lactic dehydrogenase arises from the plasma. In contrast, Dorfman, et al.² concluded that urinary enzyme did not correlate with that of serum while Plummer and Leathwood⁶ implicated kidney as the source of urinary lactic dehydrogenase rather than plasma. The origin of the lactic dehydrogenase found in the urine remains an open question.

With respect to the role of circadian cycles in response to high pressure, our results suggest that an abnormal increase in the level of enzyme activity after pressurization is related to a particular segment of the cycle, the descending phase. Other examples of in-

creased sensitivity to challenge based on diurnal rhythms are known. Halberg³ has reported circadian variations in mammalian susceptibility to doses of such agents as ethanol, ouabain, and bacterial endotoxins. Spoor and Jackson⁸ have demonstrated that the beating rate of atria isolated at 1100 hours decreased more than if isolated at 2300 hours in response to acetylcholine. In all cases cited, the reaction to stress is influenced by circadian cycles. In our situation, however, no abnormal increase is seen after an 0800-hour dive while a second peak of activity is elicited after midnight dive. Recently, a report by Krieger and Krieger⁵ described an analogous occurrence: Atropine administered to cats just prior to the time of an expected circadian rise in the levels of 17-hydroxycorticosteroid in the plasma blocks this rise. Atropine administered at other times in the circadian cycle does not alter the circadian pattern.

While dumping from sensitive cells might account for the increased plasma lactic dehydrogenase levels after a midnight dive, many other possibilities exist and must be considered. An understanding of the mechanism of the influx of enzyme from the source of storage or synthesis is absolutely essential for the interpretation of these interesting phenomena. Furthermore, the role of the specific peptide inhibitors of lactic dehydrogenase¹⁰ can not be overlooked.

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